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# Control of *Rhyzopertha dominica* in stored rough rice through a combination of diatomaceous earth and varietal resistance

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**Abstract** Adults of *Rhyzopertha dominica* (F.), the lesser grain borer, were exposed on four varieties of rough rice with Dobie indices of susceptibility of 1.1 to 1.1 (low), and four varieties with Dobie indices of susceptibility of 3.4 to 3.8 (high). The varieties with low and high Dobie indices were classified as resistant and susceptible, respectively, to *R. dominica*. The purpose of the study was to evaluate control of *R. dominica* through the use of diatomaceous earth (DE) in combination with rice varieties that were either susceptible or resistant to *R. dominica*. The rice was treated with varying rates of the commercial DE Insecto®, up to a maximum of 1 000 mg DE/kg of rice. Adult mortality at each application rate of DE was generally greater on three of four resistant varieties compared to three of four susceptible varieties. Progeny production from the parental generation exposed on the rice was also greater in 3 of the 4 resistant varieties compared to 3 of the 4 susceptible varieties at DE rates of 500 mg/kg or more. Progeny production in rice treated with a maximum rate of 1 000 mg/kg DE ranged from 7−44 adults on the resistant varieties compared to 75−155 adults on the susceptible varieties. At DE rates of 500, 750, and 1 000 mg/kg, the percentage of insect-damaged kernels (IDK) was also greater in 3/4 resistant varieties than in the susceptible varieties. Results show combining the use of DE with varietal resistance of rough rice to *R. dominica* could be used to limit populations of this insect in stored rice and help prevent economic damage.

**Key words** diatomaceous earth, DE, host plant resistance, integrated control, rice

## Introduction

*Rhyzopertha dominica* (F.), the lesser grain borer, is considered to be one of the most tolerant species of stored-grain insects to diatomaceous earth (DE), a natural inert dust registered for insect control in grain commodities (Fields & Korunic, 2000; Subramanyam & Roesli, 2000). DE efficacy is affected by formulation, particle size, grain type, and grain temperature, moisture content or relative humidity (Subramanyam & Roesli, 2000; Arthur & Throne, 2003; Arthur, 2004a). The time that insects are exposed to DE is also important because insect mortality generally increases with increases in exposure time (Arthur, 2000, 2001).

Rice is an important grain crop world-wide, and results from previous studies with rice and other grain crops show it is difficult to control *R. dominica* using DE alone (Arthur, 2004b; Chanbang *et al.*, 2007a,b). Less DE is required on brown rice or milled rice than on rough rice to control stored-grain beetles (McGaughey, 1972), which would seem to indicate some interference of the rough rice hull with the activity of DE. However, the rough rice hull may also act as a barrier to insect infestation (Chanbang *et al.*, 2008).

One possible method to improve the effectiveness of DE is to combine the DE treatment with varieties of rough rice that are resistant to *R. dominica*. In a previous experiment,

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28 different varieties of long-, short-, and medium-grain rice were evaluated for resistance to *R. dominica*, using different criteria including the Dobie Index, which is a measurement of susceptibility (Chanbang *et al*., 2008). The Dobie index was described in Chanbang *et al*. (2008), as ($\log_e$ F)/ D × 100), where F was the number of $F_1$ adults emerging from the introduction of 10 neonate *R. dominica* into each of four replicate vials containing 20 g of a particular rice variety, and D was the median development time in days of those 10 larvae. A high Dobie index indicates susceptibility to *R. dominica*, conversely a low Dobie index indicates tolerance or resistance. The four varieties that had the lowest Dobie index values for susceptibility (hereby termed "resistant") were Wells (long-grain, 1.1), Jupiter (medium-grain, 1.2), Pirogue (short-grain, 1.5), and Bengal (medium-grain, 1.6). The four varieties with the highest Dobie index values (hereby termed "susceptible") were Cocodrie (long-grain, 3.4), M-205 (medium-grain, 3.4), Akita (short-grain, 3.5), and Rico (medium-grain, 3.8). The objective of this experiment was to compare control of *R. dominica*, using different application rates of the commercial DE Insecto®, applied on these *R. dominica*-resistant and -susceptible rice varieties.

## Materials and methods

The commercial DE used in this test was Insecto®, a marine diatomaceous earth (Natural Insecto® Product, Inc., CA, USA) comprised of 90% amorphous silica and < 2.7% crystobalite. The rice varieties used in this test, as described above, were selected based on the results from Chanbang *et al*. (2008). One-kg lots of each variety were cleaned by shaking the rice over a #12 sieve (opening 1.70 mm) to remove the material and extraneous trash particles. The moisture content of each lot was determined using a Dickey-John moisture meter GAC 2000 (Dickey-John Corporation, Auburn, IL, USA). Small quantities of tap water were mixed with the rice samples until the moisture content reached 14%. Approximately 200 g of each variety were placed in a 0.95-L jar and treated individually with 0 (untreated controls), 0.05, 0.1, 0.15, and 0.2 g of DE to provide measured concentrations of 0 (untreated controls), 250, 500, 750, and 1 000 mg of DE/kg of rice, respectively. Jars were rolled horizontally in a roller machine (Norton, Akron, OH, USA) for 30 seconds to ensure uniform mixture of DE with the rice samples. Twenty grams of treated rice was sub-sampled and put in a 29-mL vial, and 20 unsexed 2-week-old *R. dominica* adults were added to the rice. The *R. dominica* adults were obtained from colonies maintained at 28 °C and 68% relative humidity (RH) on Francis variety long-grain rough rice. Voucher specimens of *R. dominica* from these colonies were previously deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research under Lot No. 162. All vials were maintained in an incubator set at 32 °C and in plastic boxes with sodium chloride solution to maintain 75% RH (Greenspan, 1977). Each treatment was repeated four times on successive days. Temperature and relative humidity were monitored during the experiment using HOBO data recorders (Onset Computer, Bourne, MA, USA).

After 2 weeks of exposure, *R. dominica* adults were sieved from vials in each treatment replicate to assess mortality. All adults were discarded and the treated rice (and untreated rice in the controls), dust from feeding damage, and insect frass were returned to the vials, which were returned to the humidity boxes and the incubator. After 56 days the rice was sieved again and *R. dominica* adult progeny were counted and then discarded. Insect damage was assessed by sampling 250 rice kernels for the presence of adult emergence holes and insect damage, termed an insect-damaged kernel (IDK).

The experimental design was a split plot, with rice variety as the main plot, and DE concentration as a subplot. Data were analyzed using the Mixed Models Procedure (PROC MIXED) of the Statistics Analysis System (SAS Institute, 2001). Mortality after the 2-week exposure, number of $F_1$ adult progeny, and the percentage of IDK were the factors analyzed in the study. Percentage mortality was transformed to angular values (Zar, 1984) while the number of progeny and number of IDK/100 kernels were transformed to square-root to normalize heteroscedastic variances. Treatment means were separated using the LSMEANS option in PROC MIXED, using the Bonferroni option to account for experiment-wise error (SAS Institute, 2001).

## Results

Mortality of parental *R. dominica* exposed on the eight rice varieties was significant ($P < 0.01$) with respect to the main effects: rice variety ($F = 31.6$; df = 7,21); the DE concentration ($F = 137.8$; df = 4,96; $P < 0.01$); and the variety × DE interaction ($F = 3.0$; df = 28,96; $P < 0.01$). In untreated rice (Table 1, 0 mg/kg DE), mortality of parental adult *R. dominica* ranged from 2.5% to 5.0% on varieties Wells, Akita, Cocodrie and M-205, and was lower than parental mortality on varieties Pirogue and Jupiter (20.2% and 52.5%, respectively). As the rate of DE increased from 250 to 1 000 mg/kg, adult mortality was generally greater in the resistant varieties Wells, Jupiter and Bengal than in the susceptible varieties Akita, Cocodrie and Rico (Table 1).

**Table 1** Percentage mortality (mean ± SE) of adult *Rhyzopertha dominica* after 14 days of exposure on different rough-rice varieties treated with 0 to 1 000 mg/kg diatomaceous earth (DE) and held at 32 °C and 75% RH.

| Rice variety | DE (mg/kg)† | | | | |
|---|---|---|---|---|---|
| | 0 | 250 | 500 | 750 | 1 000 |
| Resistant variety | | | | | |
| Bengal | 17.5 ± 6.3 ab | 67.5 ± 9.7 b | 100.0 ± 0.0 a | 98.8 ± 1.2 a | 100.0 ± 0.0 a |
| Jupiter | 52.5 ± 13.1 a | 100.0 ± 0.0 a | 100.0 ± 0.0 a | 100.0 ± 0.0 a | 100.0 ± 0.0 a |
| Pirogue | 20.0 ± 4.1 a | 12.5 ± 4.8 c | 62.5 ± 16.1 bc | 70.0 ± 10.2 bcd | 62.5 ± 13.6 bc |
| Wells | 5.0 ± 2.9 b | 48.8 ± 9.0 b | 78.8 ± 18.1 ab | 86.2 ± 10.5 abc | 97.5 ± 1.4 ab |
| Susceptible variety | | | | | |
| Akita | 2.5 ± 1.4 b | 3.7 ± 1.2 c | 28.8 ± 6.6 c | 30.0 ± 7.1 d | 46.2 ± 13.9 c |
| Cocodrie | 2.5 ± 1.4 b | 5.0 ± 0.0 c | 41.2 ± 5.9 bc | 68.8 ± 11.6 cd | 68.8 ± 9.9 bc |
| M-205 | 3.8 ± 2.4 b | 16.2 ± 2.4 c | 47.5 ± 15.6 bc | 57.5 ± 9.2 cd | 76.2 ± 7.5 abc |
| Rico | 3.8 ± 2.4 b | 13.8 ± 2.4 c | 46.2 ± 5.2 bc | 67.5 ± 9.2 cd | 63.8 ± 4.7 c |

†Means ± SE within the same DE concentration followed by different letters are significant at $P < 0.05$ (Bonferroni (Dunn) *t*-test, SAS Institute, 2001). Analyses were done on all eight varieties together.

Progeny production of *R. dominica* adults on the eight varieties was also significant with respect to the main effects: rice variety ($F = 136.5$; df = 7,21; $P < 0.01$); the DE concentration ($F = 44.3$; df = 4,96; $P < 0.01$); and the variety × DE interaction ($F = 2.3$; df = 28,96; $P < 0.01$). Progeny production on untreated rice was greater in the susceptible varieties M-205, Cocodrie and Akita, than in the susceptible varieties Jupiter, Wells and Bengal (Table 2). With the inclusion of DE, progeny production decreased differentially among the resistant and susceptible groups. In the resistant varieties treated with 250 mg/kg DE, progeny production ranged from 0 to 8.5 in resistant varieties Jupiter, Wells and Bengal, in contrast to the progeny production of 53.5 or more in the susceptible varieties. Few or no adults emerged from varieties Wells, Bengal and Jupiter treated at 500 mg/kg, while more than 49.5 adult progeny emerged from the other varieties treated at the same rate (Table 2).

The percentage of insect-damaged kernels (IDK) was significantly different ($P < 0.01$) with respect to the main effects: variety ($F = 83.4$; df = 7,21); the DE concentration ($F = 34.5$; df = 4,96); and the variety × DE interaction ($F = 2.8$; df = 28,96). In untreated rice (Table 3, 0 mg/kg DE), the percentage of IDK ranged from 2% in Jupiter to 23% in Akita. At the rate of 500 mg/kg, there were < 0.1% IDK in Wells, Bengal and Jupiter varieties, and > 5% in the others (Table 3).

**Table 2** Means ± SE number of *R. dominica* adult progeny that emerged from the different rough-rice varieties treated with 0 to 1 000 mg/kg diatomaceous earth (DE) and held at 32 °C and 75% RH for 56 days.

| Rice variety | DE (mg/kg)† | | | | |
|---|---|---|---|---|---|
| | 0 | 250 | 500 | 750 | 1 000 |
| Resistant variety | | | | | |
| Bengal | 44.5 ± 8.0 d | 8.2 ± 4.6 de | 1.0 ± 0.0 c | 0.2 ± 1.2 c | 0.0 ± 0.0 d |
| Jupiter | 7.7 ± 1.6 e | 0.0 ± 0.0 e | 0.0 ± 0.0 c | 0.0 ± 0.0 c | 0.0 ± 0.0 d |
| Pirogue | 89.0 ± 12.8 bcd | 70.0 ± 9.4 bc | 58.5 ± 17.0 b | 47.2 ± 2.5 b | 21.8 ± 2.8 bc |
| Wells | 44.0 ± 5.3 d | 2.5 ± 5.6 d | 6.0 ± 2.5 c | 6.5 ± 0.2 c | 2.0 ± 0.0 d |
| Susceptible variety | | | | | |
| Akita | 155.2 ± 22.8 a | 171.0 ± 23.3 a | 162.8 ± 6.9 a | 130.8 ± 21.7 a | 104.8 ± 14.8 a |
| Cocodrie | 135.2 ± 10.3 ab | 118.5 ± 10.4 ab | 100.2 ± 5.8 ab | 81.0 ± 14.4 ab | 74.8 ± 8.1 a |
| M-205 | 90.7 ± 5.4 abc | 53.5 ± 6.5 c | 49.5 ± 10.2 b | 47.5 ± 2.9 b | 29.5 ± 4.2 c |
| Rico | 74.5 ± 10.2 cd | 93.5 ± 9.1 bc | 51.5 ± 3.8 c | 55.0 ± 15.5 b | 48.5 ± 8.4 bc |

†Means ± SE within the same DE concentration followed by different letters are significant at $P < 0.05$ (Bonferroni (Dunn) *t*-test, SAS Institute, 2001). Analyses were done on all eight varieties together.

 

**Table 3** Percentage insect-damaged kernels caused by *R. dominica* exposed on the different rough-rice varieties treated with 0 to 1 000 mg/kg diatomaceous earth (DE) and held at 32 °C and 75% RH for 56 days.

| Rice variety | DE (mg/kg)† | | | | |
|---|---|---|---|---|---|
| | 0 | 250 | 500 | 750 | 1 000 |
| Resistant variety | | | | | |
| Bengal | 10.0 ± 2.0 c | 1.3 ± 0.5 bc | 0.5 ± 0.3 c | 0.2 ± 0.2 c | 0.1 ± 0.1 e |
| Jupiter | 1.9 ± 0.8 ab | 0.1 ± 0.1 d | 0.0 ± 0.0 c | 0.0 ± 0.0 c | 0.0 ± 0.0 e |
| Pirogue | 13.4 ± 1.6 ab | 16.2 ± 2.4 ab | 9.7 ± 1.3 b | 7.7 ± 1.3 ab | 8.7 ± 1.1 bc |
| Wells | 8.8 ± 1.2 b | 2.4 ± 1.1 bc | 0.4 ± 0.4 c | 0.5 ± 0.3 c | 0.5 ± 0.4 e |
| Susceptible variety | | | | | |
| Akita | 22.9 ± 4.4 a | 22.4 ± 3.8 a | 25.3 ± 1.1 a | 20.4 ± 1.9 a | 18.6 ± 2.3 a |
| Cocodrie | 19.1 ± 2.6 ab | 17.1 ± 3.4 ab | 14.7 ± 1.0 ab | 12.8 ± 4.8 ab | 11.3 ± 0.5 ab |
| M-205 | 19.1 ± 2.2 ab | 13.9 ± 3.6 ab | 11.2 ± 3.3 b | 8.7 ± 1.0 ab | 3.3 ± 0.5 d |
| Rico | 9.7 ± 1.0 ab | 8.1 ± 1.3 bc | 6.8 ± 0.8 b | 7.6 ± 2.9 b | 5.1 ± 1.4 cd |

†Means ± SE within the same DE concentration followed by different letters are significant at $P < 0.05$ (Bonferroni (Dunn) *t*-test, SAS Institute, 2001). Analyses were done on all eight varieties together.

## Discussion

The highest label rate of 1 000 mg/kg DE used in our study produced 46% to 100% *R. dominica* mortality, depending on the variety, with the greatest mortality in the resistant varieties Jupiter, Wells and Bengal. Other studies have repeatedly noted variation in mortality of *R. dominica* and other stored-grain insects exposed to commercial DE formulations, depending on grain type (Subramanyam *et al.*, 1994; Arthur & Throne, 2003; Arthur, 2004b; Athanassiou & Kavallieratos, 2005). Our results show that the variation among varieties of a particular grain commodity is also significant when evaluating the results of DE studies. Since DE affects adult insects primarily by absorbing and removing the cuticular wax layer, resulting in water loss (Glenn *et al.*, 1999), mortality may occur slowly, and adult females may be able to lay eggs before they die (McLaughin, 1994).

Because exposure to DE is already interfering with water loss, *R. dominica* may have additional difficulty feeding on resistant varieties. Nutritional uptake and water absorption through the diet is an important source of water for insects (Devine, 1978; Arlian, 1979), and could explain the increase in mortality in the resistant varieties treated with DE. Fewer *R. dominica* were produced on the untreated resistant varieties Wells, Jupiter and Bengal, compared to 3 of the 4 susceptible varieties. Bughio and Wilkins (2004) examined development of *Tribolium castaneum* (Herbst) from the larval to adult stages on rice flour from a resistant variety of rough rice (Dawn), a moderately resistant variety (Lebonnet), and a susceptible variety (cultivar IR8). Adult mortality of malathion-susceptible and -resistant strains of *T. castaneum* was higher on the flour from Dawn variety than other varieties, and the mortality of *T. castaneum* from rough-rice flour and milled-rice flour were higher than on brown-rice flour. The differential uptake of food material, along with the presence of seed allelochemical substances, was identified as factors that could have caused the differences in *T. castaneum* survival among the varieties tested.

Our results indicate that control through DE was enhanced on three of the four resistant varieties. Larvae of stored-product insects are extremely susceptible to DE (Subramanyam *et al.*, 1998), therefore the neonate *R. dominica* could have been more susceptible than adults to the DE. Vayias and Athanassiou (2004) reported that first instars of *T. castaneum*, the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and the Indianmeal moth, *Plodia interpunctella* (Hübner), exposed to 1 000 ppm DE on wheat were not able to emerge as adults. This larval susceptibility to DE may also enhance efficacy of DE toward neonate *R. dominica*. Combining resistant varieties with DE treatment would reduce progeny production and also decrease the percentage of IDK, as seen in our study. The percentage of IDK in the resistant varieties Wells, Jupiter and Bengal treated with 1 000 mg/kg DE was < 0.5%, while the percentage of IDK in the susceptible varieties Akita, Cocodrie, M-205 and Rico ranged from 3.3% to 18.6%.

In a previous study by Chanbang *et al.* (2008), varieties Wells, Jupiter, Bengal and Pirogue (short-grain) all had low Dobie indices for development and survival. Our results showed progeny production of *R. dominica* in untreated varieties Wells, Jupiter and Bengal was similar to progeny production in a study by Chanbang *et al*. (2008), but results for progeny production in untreated variety Pirogue were more similar to the results for the group of varieties with low Dobie indices of susceptibility, that is, the susceptible group. All of the rice used in the current study came from the same source used in Chanbang *et al*. (2008). The results for variety Pirogue were not indicative of the larger difference between the susceptible and resistant varieties, and results for the other seven varieties were consistent with the results obtained in Chanbang *et al*. (2008).

Throne *et al*. (2000) discussed varietal resistance and how it can be used to improve pest management plans for stored commodities. Our results indicate that natural or inherent resistance of rough rice to *R. dominica* could be combined with DE, which is a natural product with reduced risks to humans compared to neurotoxic grain protectants or fumigants. Since *R. dominca* is an internal feeder, damage through feeding can cause weight loss in the kernels of rough rice (Nigam *et al*., 1977), which could then result in reduced milling yields from the infested rice. Production and storage of rice varieties that may have natural resistance to *R. dominca*, and utilizing a reduced-risk insecticide, may help reduce populations of this insect species in stored rice and also help prevent damage to the commodity.

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